

Amendments to the Claims:

Please cancel Claims 1, 3 and 4 without prejudice or disclaimer, amend Claim 56, and add new Claim 137 as set forth below.

1-48. (Canceled)

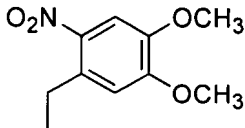
49. (Original) A substrate for a protein kinase, wherein the substrate comprises a peptide and at least one fluorophore, wherein a fluorophore is attached to a serine, a threonine, or a tyrosine on at least one terminal end of the peptide, and wherein phosphorylation by the protein kinase of the terminal serine, the terminal threonine, or the terminal tyrosine to which the fluorophore is attached produces at least a 20% change in fluorescence intensity.

50. (Original) The substrate of claim 49, wherein the substrate cannot be phosphorylated by a protein kinase until the substrate is activated.

51. (Original) The substrate of claim 50, wherein the substrate is activated by light.

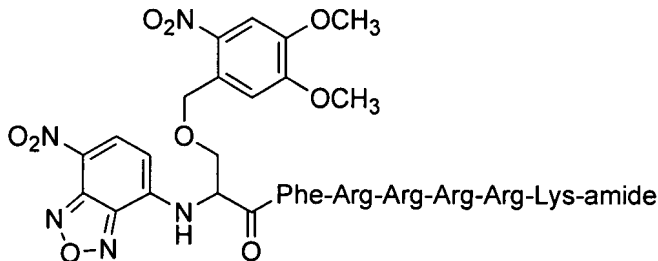
52. (Original) The substrate of claim 51, wherein the substrate comprises a serine, a threonine, or a tyrosine with a photolabile side chain that blocks transfer of a phosphoryl group from adenosine triphosphate to a hydroxyl moiety of the serine, the threonine, or the tyrosine.

53. (Original) The substrate of claim 52, wherein the photolabile side chain comprises the structure



54. (Original) The substrate of claim 52, wherein the substrate comprises a serine with a photolabile side chain that blocks phosphoryl transfer.

55. (Original) The substrate of claim 54, wherein the substrate has the structure



56. (Currently amended) A substrate for a protein kinase, wherein the substrate comprises:

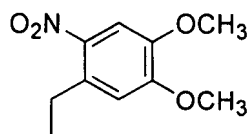
a peptide comprising a serine, a threonine, or a tyrosine on a terminal end of the peptide;

at least one fluorophore, wherein a fluorophore is attached to the serine, the threonine, or the tyrosine on the terminal end of the peptide; and

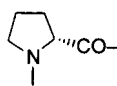
a photolabile side chain attached to the serine, the threonine, or the tyrosine on the terminal end of the peptide, wherein the photolabile side chain blocks transfer

of a phosphoryl group from adenosine triphosphate to a hydroxyl moiety of the serine, the threonine, or the tyrosine so that the substrate cannot be phosphorylated by a protein kinase until the photolabile side chain is removed from the substrate, and

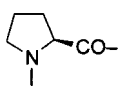
wherein the the photolabile side chain comprises the structure



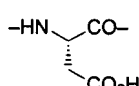
or a fluorophore is attached to the peptide by a linker selected from the group consisting of



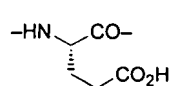
a



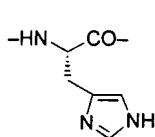
b



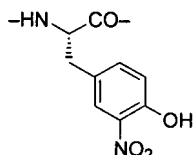
c



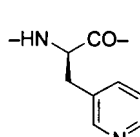
d



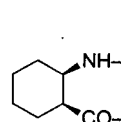
e



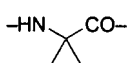
f



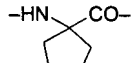
g



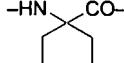
h



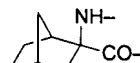
i



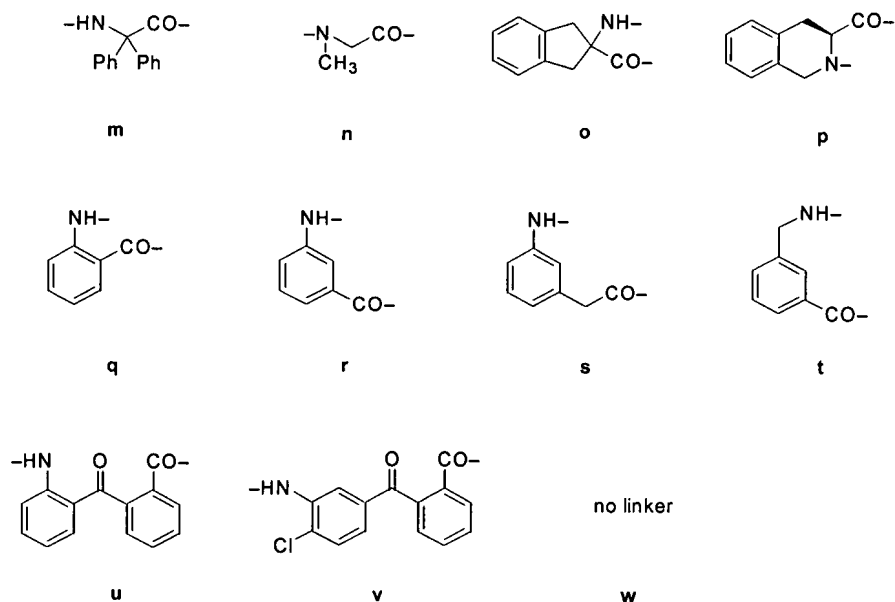
j



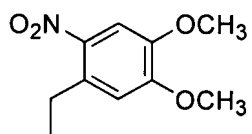
k



l

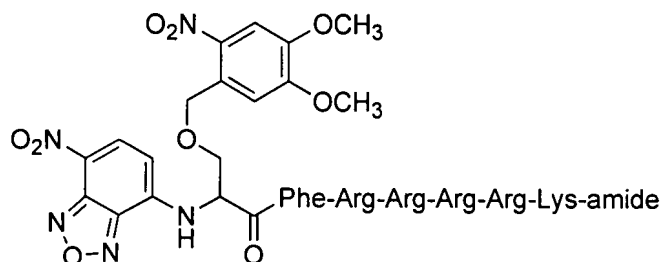


57. (Original) The substrate of claim 56, wherein the photolabile side chain comprises the structure



58. (Original) The substrate of claim 56, wherein the substrate comprises a serine with a photolabile side chain that blocks phosphoryl transfer.

59. (Original) The substrate of claim 58, wherein the substrate has the structure



60. (Original) The substrate of claim 56, wherein after removal of the photolabile side chain, phosphorylation by a protein kinase of the terminal serine, the terminal threonine, or the terminal tyrosine to which the fluorophore is attached produces at least a 20% change in fluorescence intensity.

61. (Previously presented) The substrate of claim 60, wherein the change in fluorescence intensity when the substrate is phosphorylated by the protein kinase is an increase in fluorescence intensity.

62. (Previously presented) The substrate of claim 60, wherein the change in fluorescence intensity when the substrate is phosphorylated by the protein kinase is a decrease in fluorescence intensity.

63. (Previously presented) The substrate of claim 60, wherein phosphorylation of the substrate by the protein kinase produces at least a 70% change in fluorescence intensity.

64. (Original) The substrate of claim 63, wherein phosphorylation of the substrate by the protein kinase produces at least a 100% change in fluorescence intensity.

65. (Original) The substrate of claim 64, wherein phosphorylation of the substrate by the protein kinase produces at least a 150% change in fluorescence intensity.

66. (Original) The substrate of claim 65, wherein phosphorylation of the substrate by the protein kinase produces at least a 250% change in fluorescence intensity.

67. (Previously presented) The substrate of claim 56, wherein the substrate is specific for a protein kinase subtype.

68. (Original) The substrate of claim 67, wherein the substrate is specific for protein kinase C.

69. (Original) The substrate of claim 68, wherein the substrate is specific for isoforms  $\alpha$ ,  $\beta$ , and  $\gamma$  of protein kinase C.

70. (Withdrawn) The substrate of claim 67, wherein the substrate is specific for protein kinase A, protein kinase B, protein kinase D, protein kinase G,  $\text{Ca}^{+}$ /calmodulin-dependent protein kinase, mitogen-activated protein kinase, protein kinase mos, protein kinase raf, protein tyrosine kinase, tyrosine kinase abl, tyrosine kinase src, tyrosine kinase yes, tyrosine kinase fps, tyrosine kinase met, cyclin-dependent protein kinase, or cdc2 kinase.

71. (Previously presented) The substrate of claim 56, wherein the substrate further comprises a carbohydrate, a lipid or a nucleic acid.

72. (Previously presented) The substrate of claim 56, wherein one fluorophore is

attached to one terminal end of the peptide.

73. (Original) The substrate of claim 72, wherein the fluorophore is attached to the C-terminal end of the peptide.

74. (Original) The substrate of claim 72, wherein the fluorophore is attached to the N-terminal end of the peptide.

75. (Previously presented) The substrate of claim 56, wherein a fluorophore is attached to each terminal end of the peptide.

76. (Original) The substrate of claim 75, wherein fluorophores with distinct photophysical properties are attached to different terminal ends of the peptide.

77. (Previously presented) The substrate of claim 56, wherein a first fluorophore is attached to a terminal end of the peptide and a second fluorophore, with photophysical properties distinct from the first fluorophore, is attached to any nonterminal site on the peptide.

78. (Previously presented) The substrate of claim 56, wherein the fluorophore is a 7-nitrobenz-2-oxa-1,3-diazole derivative.

79. (Withdrawn) The substrate of claim 56, wherein the fluorophore is a fluorescein derivative.

80. (Withdrawn) The substrate of claim 56, wherein the fluorophore is selected

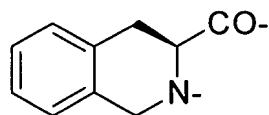
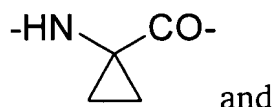
from the group consisting of a dansyl derivative, an acridine derivative, an Alexa Fluor derivative, a BODIPY derivative, an Oregon Green derivative, a Rhodamine Green derivative, a Rhodamine Red-X derivative, a Texas Red derivative, a Cascade Blue derivative, a Cascade Yellow derivative, a Marina Blue derivative, a Pacific Blue derivative, an AMCA-X derivative, and a coumarin derivative.

81. (Previously presented) The substrate of claim 56, wherein the fluorophore is attached to the peptide by a linker.

82. (Withdrawn) The substrate of claim 81, wherein the linker is a metal chelating linker.

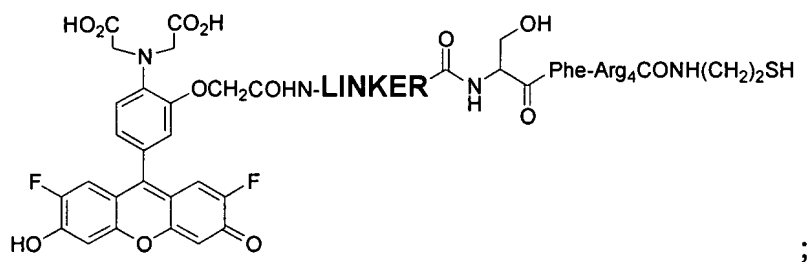
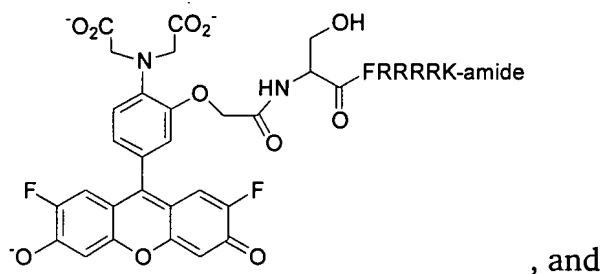
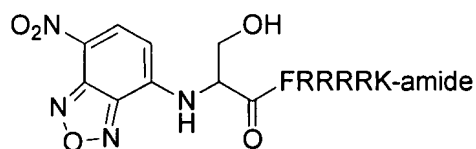
83. (Original) The substrate of claim 81, wherein the linker is selected from the group consisting of a carboxamide linker, an aminobenzoic acid linker, a sulfonamide linker, a urea linker, a thiourea linker, an ester linker, a thioester linker, an alkylamine linker, an arylamine linker, an ether linker, and a thioether linker.

84. (Withdrawn) The substrate of claim 81, wherein the linker is selected from the group consisting of N-methyl glycine, L-proline, D-proline,

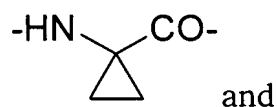


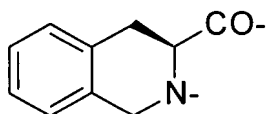


85. (Withdrawn) The substrate of claim 49, wherein the substrate is selected from the group consisting of:



wherein F is phenylalanine, K is lysine, and R is arginine; and wherein the LINKER is selected from the group consisting of N-methyl glycine, L-proline, D-proline,



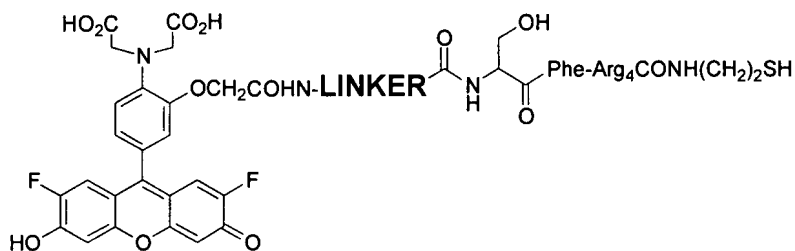


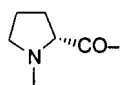
86. (Previously presented) A composition comprising the substrate of claim 56, and a carrier.

87. (Original) The composition of claim 86, wherein the composition is a pharmaceutical composition and the carrier is a pharmaceutically acceptable carrier.

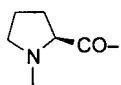
88. (Original) A chemical compound selected from the group of compounds set forth in Table 3.

89. (Original) A chemical compound having the structure:

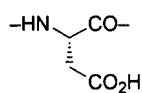




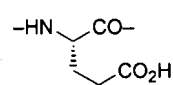
a



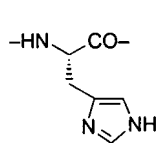
b



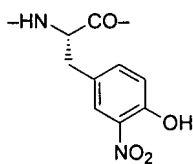
c



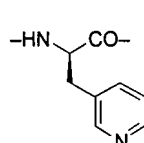
d



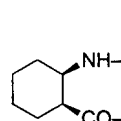
e



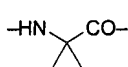
f



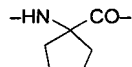
g



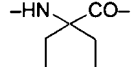
h



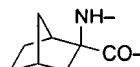
i



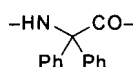
j



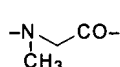
k



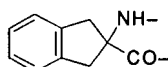
l



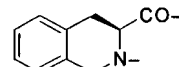
m



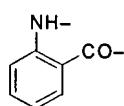
n



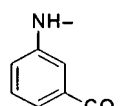
o



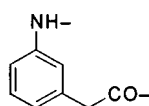
p



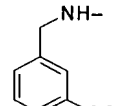
q



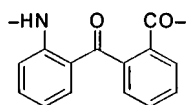
r



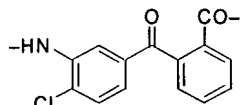
s



t



u



v

no linker

w

90. (Original) A chemical compound having the structure:

fluorophore-LINKER-X-FRRRRK-amide (SEQ ID NO:3);

wherein F is phenylalanine; K is lysine; R is arginine; and X is serine, threonine, or tyrosine.

91. (Original) The chemical compound of claim 90, wherein the fluorophore is a 7-nitrobenz-2-oxa-1,3-diazole derivative.

92. (Withdrawn) The chemical compound of claim 90, wherein the fluorophore is a fluorescein derivative.

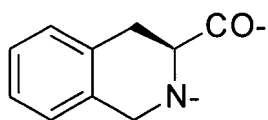
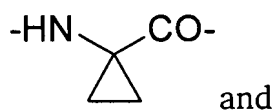
93. (Withdrawn) The chemical compound of claim 90, wherein the fluorophore is selected from the group consisting of a dansyl derivative, an acridine derivative, an Alexa Fluor derivative, a BODIPY derivative, an Oregon Green derivative, a Rhodamine Green derivative, a Rhodamine Red-X derivative, a Texas Red derivative, a Cascade Blue derivative, a Cascade Yellow derivative, a Marina Blue derivative, a Pacific Blue derivative, an AMCA-X derivative, and a coumarin derivative.

94. (Withdrawn) The chemical compound of claim 90, wherein the linker is a metal chelating linker.

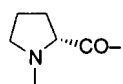
95. (Original) The chemical compound of claim 90, wherein the linker is selected from the group consisting of a carboxamide linker, an aminobenzoic acid linker, a sulfonamide linker, a urea linker, a thiourea linker, an ester linker, a thioester linker, an alkylamine linker, an arylamine linker, an ether linker, and a thioether linker.

96. (Withdrawn) The chemical compound of claim 90, wherein the linker is

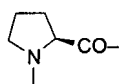
selected from the group consisting of N-methyl glycine, L-proline, D-proline,



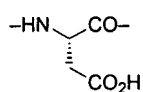
97. (Original) The chemical compound of claim 90, wherein the linker is selected from the group consisting of the following:



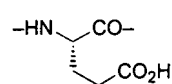
a



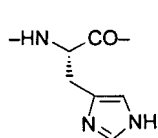
b



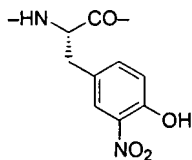
c



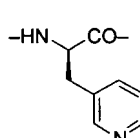
d



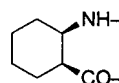
e



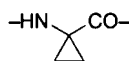
f



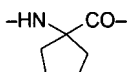
g



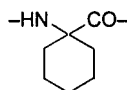
h



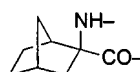
i



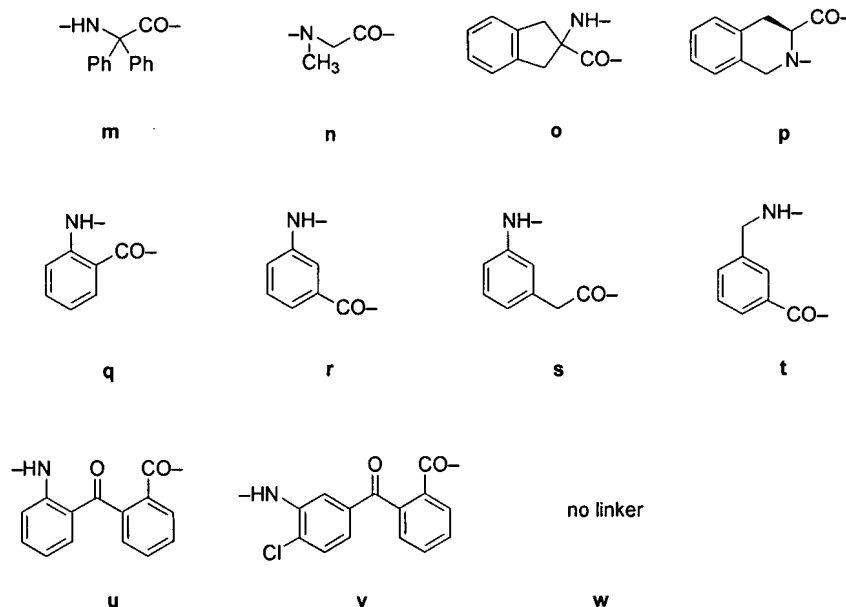
j



k



l



98. (Original) The chemical compound of claim 90, wherein the chemical compound is a substrate for a protein kinase.

99. (Original) The chemical compound of claim 98, wherein the chemical compound is specific for protein kinase C.

100. (Original) The chemical compound of claim 99, wherein the chemical compound is specific for isoforms  $\alpha$ ,  $\beta$ , and  $\gamma$  of protein kinase C.

101. (Withdrawn) The chemical compound of claim 98, the chemical compound is specific for protein kinase A, protein kinase B, protein kinase D, protein kinase G,  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase, mitogen-activated protein kinase, protein kinase mos, protein kinase raf, protein tyrosine kinase, tyrosine kinase abl, tyrosine kinase

Applicant: David S. Lawrence  
Serial No.: 10/755,086  
Filed: January 9, 2004  
page 16 of 27

src, tyrosine kinase yes, tyrosine kinase fps, tyrosine kinase met, cyclin-dependent protein kinase, or cdc2 kinase.

102. (Original) The chemical compound of claim 90, wherein the chemical compound further comprises a carbohydrate, a lipid or a nucleic acid.

103. (Original) A chemical compound comprising a peptide and at least one fluorophore, wherein a fluorophore is attached to a serine, a threonine, or a tyrosine on at least one terminal end of the peptide.

104. (Original) The chemical compound of claim 103, wherein the fluorophore is attached to the C-terminal end of the peptide.

105. (Original) The chemical compound of claim 103, wherein the fluorophore is attached to the N-terminal end of the peptide.

106. (Original) The chemical compound of claim 103, wherein a fluorophore is attached to each terminal end of the peptide.

107. (Original) The chemical compound of claim 106, wherein fluorophores with distinct photophysical properties are attached to different terminal ends of the peptide.

108. (Original) The chemical compound of claim 103, wherein a first fluorophore is attached to a terminal end of the peptide and a second fluorophore, with photophysical properties distinct from the first fluorophore, is attached to any nonterminal site on the peptide.

109. (Original) The chemical compound of claim 103, wherein the fluorophore is a 7-nitrobenz-2-oxa-1,3-diazole derivative.

110. (Withdrawn) The chemical compound of claim 103, wherein the fluorophore is a fluorescein derivative.

111. (Withdrawn) The chemical compound of claim 103, wherein the fluorophore is selected from the group consisting of a dansyl derivative, an acridine derivative, an Alexa Fluor derivative, a BODIPY derivative, an Oregon Green derivative, a Rhodamine Green derivative, a Rhodamine Red-X derivative, a Texas Red derivative, a Cascade Blue derivative, a Cascade Yellow derivative, a Marina Blue derivative, a Pacific Blue derivative, an AMCA-X derivative, and a coumarin derivative.

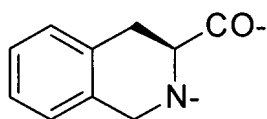
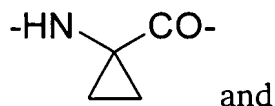
112. (Original) The chemical compound of claim 103, wherein the fluorophore is attached to the peptide by a linker.

113. (Withdrawn) The chemical compound of claim 112, wherein the linker is a metal chelating linker.

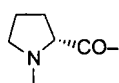
114. (Original) The chemical compound of claim 112, wherein the linker is selected from the group consisting of a carboxamide linker, an aminobenzoic acid linker, a sulfonamide linker, a urea linker, a thiourea linker, an ester linker, a thioester linker, an alkylamine linker, an arylamine linker, an ether linker, and a thioether linker.



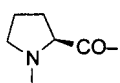
115. (Withdrawn) The chemical compound of claim 112, wherein the linker is selected from the group consisting of N-methyl glycine, L-proline, D-proline,



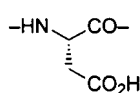
116. (Original) The chemical compound of claim 112, wherein the linker is selected from the group consisting of the following:



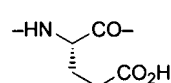
a



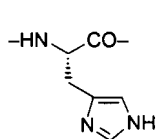
b



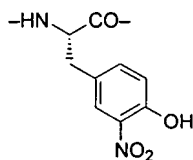
c



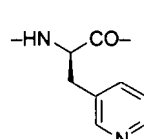
d



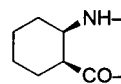
e



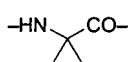
f



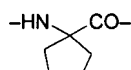
g



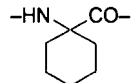
h



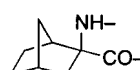
i



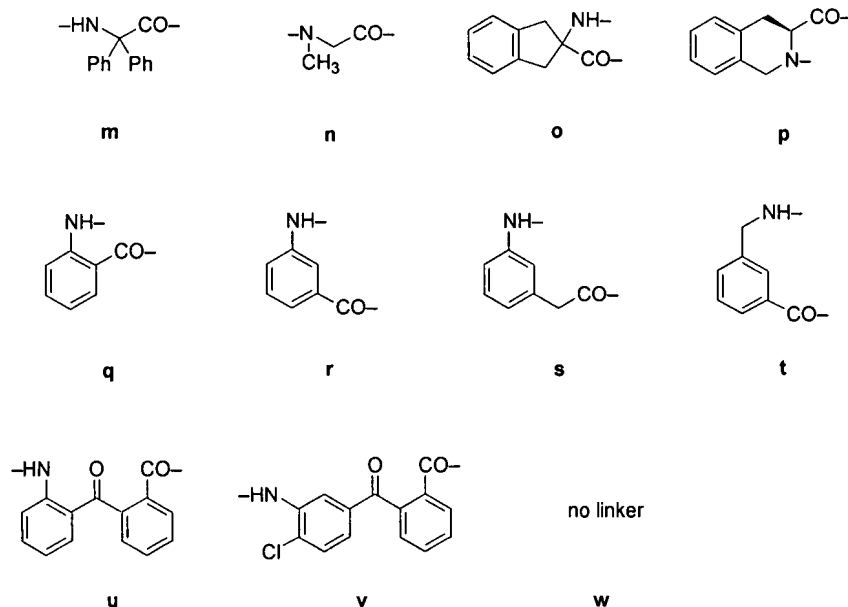
j



k



l



117. (Original) The chemical compound of claim 103, wherein the chemical compound is a substrate for a protein kinase.

118. (Original) The chemical compound of claim 117, wherein the chemical compound is specific for protein kinase C.

119. (Original) The chemical compound of claim 118, wherein the chemical compound is specific for isoforms  $\alpha$ ,  $\beta$ , and  $\gamma$  of protein kinase C.

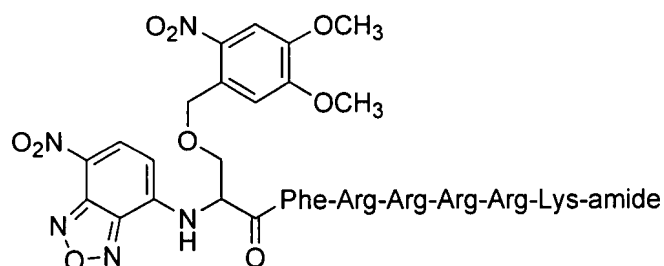
120. (Withdrawn) The chemical compound of claim 117, wherein the chemical compound is specific for protein kinase A, protein kinase B, protein kinase D, protein kinase G,  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase, mitogen-activated protein kinase, protein kinase mos, protein kinase raf, protein tyrosine kinase, tyrosine kinase abl, tyrosine kinase src, tyrosine kinase yes, tyrosine kinase fps, tyrosine kinase met, cyclin-

Applicant: David S. Lawrence  
Serial No.: 10/755,086  
Filed: January 9, 2004  
page 20 of 27

dependent protein kinase, or cdc2 kinase.

121. (Original) The chemical compound of claim 103, wherein the chemical compound further comprises a carbohydrate, a lipid or a nucleic acid.

122. (Original) A chemical compound having the structure



123. (Previously presented) A composition comprising a chemical compound of claim 89, and a carrier.

124-126. (Canceled)

127. (Previously presented) The substrate of claim 60, wherein a metal ion chelator induces the change in fluorescence intensity.

128. (Original) The substrate of claim 127, wherein the metal ion is a magnesium ion or a calcium ion.

129. (Previously presented) The chemical compound of claim 94, wherein a metal ion chelator induces a change in fluorescence intensity.

130. (Original) The chemical compound of claim 129, wherein the metal ion is a magnesium ion or a calcium ion.

131. (Original) The chemical compound of claim 129, wherein the change in fluorescence intensity is at least a 20% change in fluorescence intensity.

132. (Canceled)

133. (Original) The substrate of claim 81, wherein the linker comprises a turn to position the fluorophore in a location closer to the terminal serine, the terminal threonine or the terminal tyrosine than the location the fluorophore would occupy in the absence of a turn in the linker.

134. (Previously presented) The chemical compound of claim 89, wherein the linker comprises a turn to position the fluorophore in a location closer to the terminal serine, the terminal threonine or the terminal tyrosine than the location the fluorophore would occupy in the absence of a turn in the linker.

135-136. (Canceled)

137. (New) The composition of claim 123, wherein the composition is a pharmaceutical composition and the carrier is a pharmaceutically acceptable carrier.